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IMMUNOHISTOCHEMISTRY

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The thought of being able in one way or another to localize antigen-antibody reactions in tissue preparations for light-microscopic observations is old. Most attempts have consisted in marking the antigen or antibody with the usual stains. Such experiments have met with many difficulties, of which the most substantial one has been that such marking requires so much dye that the reactive groups in antigen or antibody are blocked. Thereby the biological and immunological qualities of such substances are lost to a large extent.

It was therefore a very important step forward when Coons et al (1) introduced the use of fluorescent substances in immunohistochemistry. First because antibodies can be combined with fluorescent material without losing their ability to react with the antigen in question. Secondly because such marked antibody and therewith the antigen-antibody complex, can be observed in tissue sections by means of fluorescence microscopy. For this purpose the preparation is irradiated with ultraviolet light in a dark field. By such irradiation fluorescent materials become luminous and can thereby be observed and localized in the tissue section.

In the use of this method a number of technical conditions assert themselves however (5).

As regards the treatment of the tissue that is to be studied, the usual methods for fixing or dehydration cannot be used. The fact is that these involve a danger of diffusion, of the loss of the immunological qualities of many antigens and of artifact formations that can make interpretation of the observations difficult. Techniques have therefore been introduced that aim at treating the tissue as gently as possible. The most common are the cryostat method and the freeze-drying method.

In both cases the tissue is frozen in liquid nitrogen (-180°C). In the freeze-drying method the tissue is tried in vacuo at ca -35° C. Next it is molded in vacuo and it is then ready for slicing. By the cryostat method the deep-frozen tissue is transferred directly to a microtome placed in a freezing cabinet where the temperature is ca -20°C. Next the sections are cut and can then be used directly, or after drying, for immunohistochemical studies.

The quickest of these methods is the cryostat method, which also permits work to be done with larger pieces of tissue than is the case in material for freeze-drying. By the cryostat method distortions and the formation of artifacts can occur during the slicing, however, as also diffusions can occur when the section dries. Freeze-drying is more cumbersome, but in return yields more reliable results as regards localization and cytological details.

In the following immunohistochemical studies either the direct or the indirect method is used.

By the direct method the section is covered with fluorescent antibody and incubated for 20 minutes for antibody to be bound to antigen. Next the sections are thoroughly washed to remove foreign matter and excess antibody. The sections are then conveyed through ethanol and xylol in the usual manner and imbeded in Fluormount.

By the indirect method two treatments of the sections are used with different antibodies. First an unmarked antibody is placed on the section, which antibody was formed in a test animal against the tissue antigen in which one is interested. The specific antibody will thereby bind itself to its antigen. After washing the section is covered with fluorescent antiserum produced against gamma globulin from the species of animal that was used for production of the first unmarked antiserum. Thereupon the section is treated as with the direct method.

There are two advantages in the indirect method. First, a considerably stronger fluorescence is obtained than with the direct method. Second, it is not necessary to prepare fluorescent antibody against every single antigen one is interested in. There are also disadvantages however, in that various types of unspecific reactions may occur, so careful control stainings must be carried out.

Production and isolation of antibodies can offer difficulties of various kinds. If the experiment requires the production of antibody by injection of a specific antigen in a test animal it is, aside from the dosage, important that the antigen be chemically pure. This often makes it necessary to use partly complicated chemical and biochemical methods based on various types of modern apparatus. Commonly used techniques include various forms of chromatography, electrophoresis and gel filtration.

As far as the isolation of antibody from serum is concerned, this can be done in several ways. At the present the most common consists of a chemical precipitation of gamma globulin, possibly followed by a further cleaning by methods of the type mentioned above.

Control of the degree of purity of antigen and antibody can then be undertaken by means of agar diffusion or immunoelectrophoresis.

Conjugation with fluorescence takes place by means of chemical reactions which previously could be quite difficult. These have now become much simpler after the introduction of fluorescein isothiocyanate (FITC) which is a chemically stable substance and which combines with antibody in a satisfactory manner.

The form of immunohistochemical technique outlined here has found wide utilization, among other things for histological localization of protozoa, bacteria and virus as well as in regard to isolated microbial antigens. The method has also been used to localize several types of protein, e.g. plasma proteins, enzymes and hormone proteins. The same is true of compound proteins, a group of substances including among others the blood group substances. In this connection it is of interest that by means of the fluorescence technique it has been possible to show that complements are bound to the antigen-antibody complex.

Until now the fluorescence technique has been used less for diagnostic purposes. A rapid development in this area must however be expected, especially as regards quick bacteriological and virological diagnosis. In addition there is reason for believing that fluorescence methods may offer great advantages with regard to diagnosis of diseases connected with the existence of autoimmunity. Among these latter belong, as we know, the group of diseases known under the designation of "collagen diseases".

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